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Turkey and chicken interferon-γ, which share high sequence identity, are biologically cross-reactive

Shelly Lawson[a,*], Lisa Rothwell[a], Benedicte Lambrecht[b], Ken Howes[a], K. Venugopal[a], Pete Kaiser[a]

[a] *Institute for Animal Health, Compton, Berkshire, RG20 7NN, UK*
[b] *Section of Avian Virology and Biotechnology, Veterinary and Agrochemical Research Centre (VAR), Groeselenberg 99, B 1180 Brussels, Belgium*

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Abstract

Turkey and chicken interferon-γ (IFN-γ) share high identity (96.3% and 97% at the nucleotide and amino acid level, respectively). As such, we predicted that they would be functionally cross-reactive. To test this hypothesis, we produced recombinant turkey and chicken IFN-γ, and compared their biological properties. Recombinant turkey and chicken IFN-γ both induce HD11 cells (a chicken macrophage cell line) and LSTC-IAH30 cells (ALV-J-transformed turkey macrophages) to produce nitric oxide (NO), as measured in an avian IFN-γ bioassay. Polyclonal and monoclonal antibodies, capable of neutralising the effect of chicken IFN-γ on HD11 cells, were also shown to inhibit the activity of turkey IFN-γ on these cells. The antibody neutralisation effect on both turkey and chicken IFN-γ was shown by a significant reduction in NO production by HD11 cells when the neutralising antibodies were present in the bioassay. FACS analysis showed that HD11 and LSTC-IAH30 cells share some cell surface markers.

Keywords: IFN-γ; Cross-reactivity; Chicken; Turkey; Macrophages; Bioassay

Abbreviations: aa, Amino acid; Ab, Antibody; ALV, Avian leukosis virus; bp, Base pairs; BSA, Bovine serum albumin; cDNA, Complementary deoxyribonucleic acid; ConA, Concanavalin A; CS, Chick serum; DMEM, Dulbecco's Modified Eagle's Medium; DNA, Deoxyribonucleic acid; dNTP, Deoxynucleotide triphosphate; ELISA, Enzyme-linked immunosorbent assay; FACS, Fluorescence activated cell sorting; FBS, Foetal bovine serum; FITC, Fluorescein isothiocyanate; IAH, Institute for Animal Health; IFN, Interferon; IL, Interleukin; LB, Luria-Bertani Broth; LPS, Lipopolysaccharide; mAb, Monoclonal Ab; mRNA, Messenger RNA; NO, Nitric oxide; nt, Nucleotide; P/S, Penicillin/streptomycin; pAb, Polyclonal Ab; PBL, Peripheral blood leukocytes; PBS, phosphate buffered saline; PCR, Polymerase chain reaction; PHA, Phytohaemagglutinin; PMA, Phorbol 12-myristate 13-acetate; PWM, pokeweed mitogen; RT-PCR, reverse transcriptase-PCR; RNA, Ribonucleic acid; TPB, Tryptose phosphate broth; U, Units.

* Corresponding author. Tel.: +44-1635-578411; fax: +44-1635-577263.
E-mail address: michelle.lawson@bbsrc.ac.uk (S. Lawson).

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1. Introduction

At present the cDNAs for only three turkey cytokines have been cloned: type I IFN [1], IFN-γ [2] and IL-2 [3]. Turkey type I IFN shares 91% and 82% identity with chicken type I IFN at the nucleotide (nt) and amino acid (aa) sequence levels, respectively. Chicken and turkey type I IFN have also been shown to be cross-reactive [1]. Turkey and chicken IL-2 share 86.2% and 69.9% identity at the nt and aa sequence levels respectively and are less conserved than IL-2 from closely related mammalian species [3]. Despite this low degree of identity at the aa level, turkey and chicken IL-2 are cross-reactive. In comparison, turkey IFN-γ (TuIFN-γ) shares 96.3% and 97% identity with chicken IFN-γ (ChIFN-γ) at the nt and aa sequence levels, respectively [2]. The high degrees of identity seen among the galliforms for IFN-γ (93.5–96.7% and 87.8–96.6% at the nt and aa levels, respectively) suggested cross-species reactivity [2]. To test this hypothesis, recombinant IFN-γ from turkey and chicken were produced in order to compare their biological properties.

IFN-γ is a pleiotropic cytokine involved in the regulation of nearly every stage of immune and inflammatory responses. It is associated primarily with a T helper 1 immune response, driving cell-mediated immunity. IFN-γ augments the expression of MHC class II antigens on many different accessory cells, thus stimulating the interaction of these cells with T cells, promoting antibody (Ab) IgG2a isotype switching and the development of cytotoxic T cells. Its primary function is to activate resting macrophages, which once activated kill pathogens within cells. Macrophages activated by IFN-γ produce reactive nitrogen species, including nitric oxide (NO) [4]. We used this property to measure the ability of recombinant ChIFN-γ (rChIFN-γ) and recombinant TuIFN-γ (rTuIFN-γ) to stimulate turkey or chicken macrophages.

2. Materials and methods

2.1. Animals

Big 6 line turkeys (British United Turkeys) were obtained at hatch from Sun Valley Foods Ltd., Ludlow, Shropshire, UK, and maintained at IAH. Chickens (Sykes Rhode Island Reds) were produced and maintained at IAH.

2.2. Culture of the turkey macrophage cell line, LSTC-IAH30, and the chicken macrophage cell line, HD11

Turkey macrophages were isolated from peripheral blood of four week-old poults. 10 ml of heparinised blood was centrifuged over Histopaque 1083 (Sigma, Poole, UK) at 1200 × *g* for 40 min at 4°C and the leukocyte fraction collected. Cells were washed three times in Dulbecco's Modified Eagle's Medium (DMEM) (Life Technologies, Paisley, UK) and then counted. 5×10^6 leukocytes were seeded into 50 mm petri dishes in 5 ml of growth media (an equal volume of Leibovitz L15 medium (Life Technologies) and McCoy's 5A medium (Life Technologies) containing 5% fetal bovine serum (FBS) and 5% chick serum (CS)) and incubated at 38.5°C in 5% CO_2. The medium was changed each day for 4 days. The cells were then challenged with 10^3 chicken bone marrow cell-transforming units of acutely transforming avian leukosis virus (ALV) strain 966 [5], absorbed for 1 h at 38.5°C in 5% CO_2. Fresh medium was then added. The medium was then changed every 2–3 days until the cultures were completely transformed (10–14 days). The transformed cells were then maintained by subculture. Every 3–4 days, cells were passaged, using standard conditions [6] and seeded at 4×10^4 ml^{-1} of growth media (an equal volume of Leibovitz L15 medium and McCoy's 5A medium, containing 8% FBS, 2% CS, 5% tryptose phosphate broth (TPB), sodium pyruvate (100 mM), 1 U/ml penicillin and 1 μg/ml streptomycin), changing the media every 2 days. At intervals extracts of the cells were tested for ALV group specific antigen by capture ELISA and for acutely transforming virus in the supernatant, by chicken bone marrow cell transformation. All tests were positive (results not shown). The resulting turkey macrophage cell line, LSTC-IAH30, was cultured in an equal volume of Leibovitz L15 medium and McCoy's

5A medium, containing 8% FBS, 2% CS, 5% TPB, 100 mM sodium pyruvate, 1 U/ml penicillin and 1 μg/ml streptomycin (P/S). Every 2 days, cells were passaged and seeded at 4×10^4 ml^{-1}.

HD11 cells, an ALV virus (MC29) transformed chicken macrophage cell line [7], were cultured in RPMI 1640 (Life Technologies) containing 2.5% FCS, 2.5% CS, 10% TPB, 20 mM L-glutamine and P/S. Every 3–4 days cells were passaged and seeded at 4×10^5 ml^{-1}.

2.3. Cloning and analysis of TuIFN-γ and ChIFN-γ cDNA

mRNA was isolated from splenocytes from 3- to 5-week-old turkey poults. Spleens were collected aseptically in DMEM and tissue was broken up with sterile forceps. Leukocyte cell suspensions were isolated by centrifugation over Ficoll-Paque (Amersham Pharmacia Biotech, St Albans, UK) at $1000 \times g$ for 20 min at 4°C and washed twice in DMEM. The cells were resuspended at 1×10^7 ml^{-1} in DMEM containing 1% L-glutamine, 2 mg ml^{-1} BSA and P/S. Cells were stimulated with 0.8 μg ml^{-1} of PMA (Sigma) and incubated at 41°C in 5% CO_2. Cells were harvested and mRNA isolated at 6, 12, 18 and 24 h after PMA stimulation, using an Oligotex Direct Midi kit (Qiagen, Crawley, UK). RT-PCR were performed to obtain turkey and chicken IFN-γ cDNAs, encoding the signal sequence and mature protein (including the stop codon), using primers designed by Kaiser et al. [2] to the chicken genomic IFN-γ sequence (Accession No. Y07922). Primer IFN3 (5′ATGACTTGCCAGACTTACAA 3′) corresponds to nt positions 1–20 of the coding sequence. Primer IFN4 (5′TTACGAATTGCATCTCCTCT 3′) is reverse complementary to nt positions 495–475 in the cDNA.

First strand synthesis was for 2 h at 42°C in a 20 μl volume, containing 4 pmol of the reverse primer (IFN4), 200 U Superscript II (Life Technologies) and 500 ng mRNA. After denaturation of the polymerase at 94°C for 4 min, 10 μl of this reaction mix was added as DNA template to a 50 μl standard PCR, containing 20 pmol of each primer (IFN3 and IFN4) and 2.5 U *Taq* polymerase (Life Technologies). Cycling conditions were 94°C for 1 min, 55°C for 2 min, 72°C for 2 min, for 25 cycles, using a Hybaid TouchDown PCR machine. The resulting PCR product was ligated into pGEM-T Easy vector (Promega, Southampton, UK) and the complete sequence of five representative clones determined on each strand using the PRISM® Ready Reaction Dye-Deoxy® Terminator cycle sequencing kit (Applied Biosystems, Warrington, UK). Sequence data was analysed with the Wisconsin Package software (Genetics Computer Group; [8]). The cDNA inserts were then ligated into the *Not*I site of the expression vector pCIneo (Promega). The resulting constructs were sequenced to confirm that the IFN-γ cDNAs were error-free and in the correct orientations.

2.4. Expression of recombinant TuIFN-γ and ChIFN-γ

COS-7 cells were routinely grown in DMEM (containing 10% FBS, 1% non-essential aa, 1% L-glutamine and P/S) at 37°C in 5% CO_2 and passaged using standard conditions [6]. Cells were cultured at 5×10^5 ml^{-1} for 18–24 h at 37°C in 5% CO_2, and washed twice with PBS. 5 ml of serum-free media was added, containing 7.5 μg ml^{-1} DNA (pCIneo containing either TuIFNγ or ChIFN-γ (pTuIFNγ or pChIFNγ, respectively), pCIneo alone or no plasmid), 258 μg ml^{-1} chloroquine and 600 μg ml^{-1} DEAE-dextran. Flasks were incubated for 3 h at 37°C in 5% CO_2. The transfection media were then removed and the cells washed once with PBS. PBS containing 10% dimethyl sulphoxide was then added for 2 min, removed and replaced with 5 ml of growth media. After 24 h growth at 37°C in 5% CO_2, growth media was replaced with serum-free growth media. The cells were then incubated for 72 h, following which supernatant was collected and stored at 4°C prior to use in the IFN-γ bioassays.

2.5. Bioassay and neutralisation assay

Immediately before use in the IFN-γ bioassay, cells (LSTC-IAH30 and HD11) were

trypsinised, adjusted to 5×10^5 ml^{-1} and added (100 μl/well) to 96-well flat-bottomed plates (Costar, Corning, NY, USA). Serial dilutions of supernatants from transfected COS-7 cells, as described above, were added, in triplicate, to give a final volume of 200 μl/well. Negative controls included supernatants collected from COS-7 cells transfected with pCIneo alone, or no plasmid. Lipopolysaccharide (LPS — *E. coli* serotype 055:B5) (Sigma) was used as a positive control, with ten-fold serial dilutions starting at 10 μg ml^{-1}. All plates were then incubated at 41°C in 5% CO_2 for 48 h.

An IFN-γ neutralising assay was set up using either HD11 or LSTC-IAH30 cells at the concentrations described in the IFN-γ bioassay. Anti-chicken IFN-γ neutralising Abs, mAb 1E-12 [9] and a rabbit polyclonal Ab 88 (kindly provided by J. Lowenthal, CSIRO, Australia), were diluted 1:100 in HD11 or LSTC-IAH30 cell media. 50 μl/well of Ab were added to 96-well flat-bottomed plates in triplicate and mixed with 50 μl of supernatants from transfected COS-7 cells, as described above, or LPS (diluted as described above). An isotype matched anti-bovine CD4 mAb CC30 [10], a polyclonal goat anti-mouse IgG FITC conjugate (Cambridge Bioscience, UK) and medium alone were used as negative controls. After 1 h incubation at 4°C, 100 μl of HD11 or LSTC-IAH30 cells were added to each well and incubated at 41°C in 5% CO_2 for 48 h.

Nitric oxide production from HD11 or LSTC-IAH30 cells in the IFN-γ bioassay or IFN-γ neutralising bioassay was measured using a modification of the Griess assay. Equal volumes of 1% sulfanilamide and 0.3% naphthylethylenediamine, in 2.5% H_3PO_4, were mixed and 100 μl/well added to 96-well flat-bottomed plates. 100 μl/well of the supernatants from the incubated HD11 or LSTC-IAH30 cells were added to the substrate. Absorbance at 543 nm was read on a Spectra Max 250 ELISA reader (Molecular Devices, Wokingham, UK).

For the neutralising bioassay, the results were examined by one-way analysis of variance to identify if there were any significant differences between the treatments. A Tukey test for multiple comparison of K population means was then used to identify which treatments were significantly different.

2.6. Capture ELISA

Splenic cell suspensions from chicken and turkeys were prepared essentially as described above, and resuspended at 1×10^7 ml^{-1} in DMEM containing 1% L-glutamine, 2 mg ml^{-1} BSA and P/S. Cells were stimulated with various concentrations of ConA, LPS (in this assay, *E. coli* serotype 0111:B4), PHA or PWM (all Sigma) and incubated for 48 h at 41°C in 5% CO_2. The resulting supernatants were assayed for IFN-γ content using a quantitative capture ELISA, as described [9].

2.7. FACS analysis

Splenic cell suspensions from turkeys were prepared essentially as described above. PBLs were isolated from 5 ml of heparinised blood by centrifugation at 4°C for 15 min at $2100 \times g$ over Ficoll-Paque and washed twice in DMEM. HD11 and LSTC-IAH30 cells were passaged (as described above). All cell suspensions were adjusted to 2×10^7 ml^{-1} in cold PBS containing 2 mg ml^{-1} BSA and 0.1% sodium azide (PBS/BSA/Azide).

Fifty microlitres of cells were added per well to round-bottomed 96-well plates (Nunclon, Nunc, Denmark) and centrifuged at 4°C for 2 min at $150 \times g$. Supernatants were removed and cells resuspended in 25 μl of each of the different anti-chicken mAb [10–17] (see Table 1) at optimum dilutions in PBS/BSA/Azide. PBS/BSA/Azide alone and CC30 were used as negative controls. Plates were incubated at 4°C for 45 min. Cells were washed twice in 100 μl of PBS/BSA/Azide, resuspended in 25 μl of goat anti-mouse IgG conjugated to FITC (Cambridge Bioscience) at a 1:200 dilution and incubated at 4°C for 45 min. The wash was repeated in 100 μl of PBS/BSA/Azide and cells resuspended in 400 μl of sheath fluid (normal saline). Each sample was analysed on a FACScan (Becton Dickinson, Oxford, UK).

The fluorescence (FL1) of 5000 cells in each sample was measured.

3. Results

3.1. Turkey and chicken IFN-γ cross-react

Turkey and chicken IFN-γ cDNAs (encoding the signal sequence and mature protein, including the stop codon) were cloned separately into the expression vector pCIneo and the resulting constructs (pTuIFN-γ and pChIFN-γ respectively) sequenced to confirm the DNA insert was error-free and in the correct orientation (results not shown). Recombinant IFN-γ was then produced by transfecting COS-7 cells with pTuIFN-γ or pChIFN-γ, and collecting supernatant after 72 h incubation at 37°C in 5% CO_2. Controls included COS-7 cells transfected with pCIneo alone, and mock-transfected COS-7 cells. Identical supernatants were tested in all bioassays.

The species-specific activities of rTuIFN-γ and rChIFN-γ were examined in an IFN-γ bioassay [7,18]. The assay works on the principle that IFN-γ activates macrophages to aid the killing of intracellular pathogens in infected cells, by the production of reactive nitrogen species, including NO [4]. Here we measured the ability of rIFN-γ to induce chicken macrophages (HD11 cell line [7]) or turkey macrophages (LSTC-IAH30 cell line) to secrete NO, as measured by nitrite accumulation in the culture supernatants. LPS (*E. coli* serotype 055:B5) also stimulates chicken macrophages to produce NO and was used as a positive control. For all samples, triplicate IFN-γ bioassays were performed. For HD11 cells (Fig. 1(A)), both rTuIFN-γ and rChIFN-γ induced similar levels of NO. Neither the supernatants from mock-transfected COS-7 cells, nor those from COS-7 cells transfected with pCIneo alone, stimulated significant NO production. LPS also induced HD11 cells to produce significant levels of NO.

In case of LSTC-IAH30 cells (Fig. 1(B)), similar results were seen for rTuIFN-γ and rChIFN-γ. The level of NO produced by LSTC-IAH30 cells was far less than that produced by HD11 cells. However, NO production from LSTC-IAH30 cells, stimulated by rTuIFN-γ and rChIFN-γ, was significantly greater than that seen from the negative controls. One surprising result was that LPS did not induce NO production from LSTC-IAH30 cells (Figs. 1(B) and 3). However, this was later shown to be a serotype-specific result, as stimulation with LPS from *E. coli* serotype 055:B26 caused NO production by LSTC-IAH30 cells (results not shown).

A quantitative capture ELISA developed to measure ChIFN-γ [9] was also shown to measure TuIFN-γ. Supernatants from chicken (Fig. 2(A)) or turkey (Fig. 2(B)) splenocytes, cultured for 48

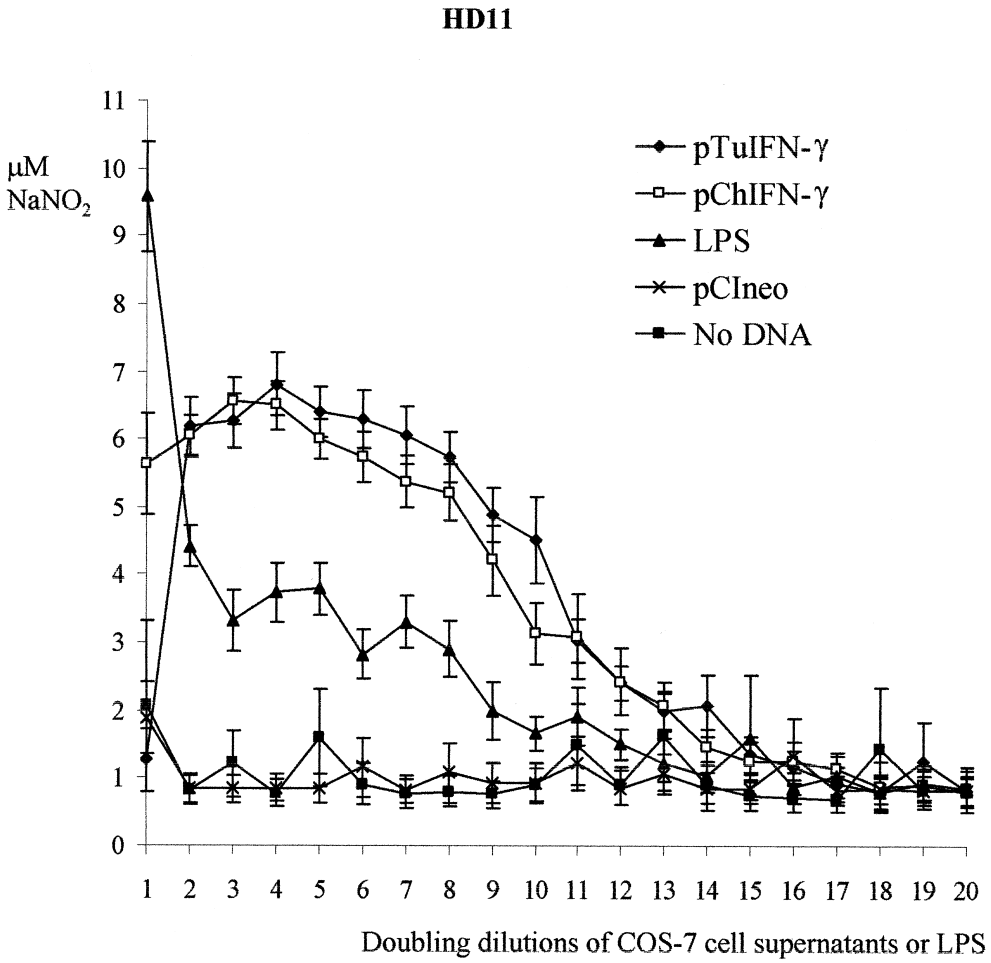
Table 1
Monoclonal antibodies used in FACS analysis

Monoclonal antibody	Antigen specificity	Reference/source
CC48	Undefined bovine antigen	Howard, IAH
CC30	Bovine CD4	[10]
AV6	CD44	Davison, IAH
AV29	CD4	Davison, IAH
3-298	CD8α	[11]
TCR2	TCRαβ_1	Southern Biotechnology
AV7	CD28	[12]
M1	IgM	[13]
K1	Thrombocyte/macrophage marker	[14]
KULO1	Macrophage marker	[15]
F21-21	β_2M	[16]
2G11	MHC class II	[17]

h in the presence of various mitogens, were tested for IFN-γ content using the ELISA. Supernatants from LPS-treated splenocytes were added as a positive control. Both chicken and turkey splenocytes were stimulated to produce IFN-γ, as measured by the capture ELISA, following mitogen stimulation.

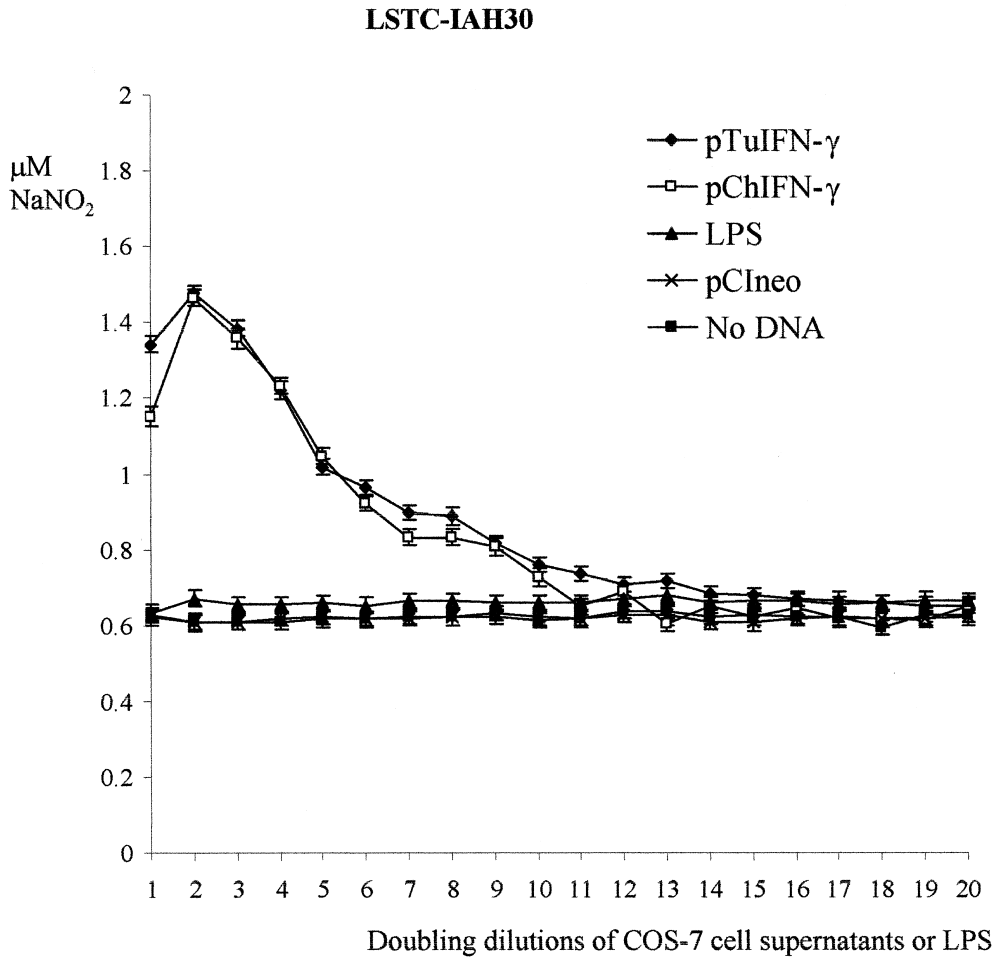
3.2. The biological activity of turkey IFN-γ is neutralised by mAb and pAb specific for chicken IFN-γ

To further illustrate the biological similarity of TuIFN-γ and ChIFN-γ, an IFN-γ neutralising assay was carried out. The anti-chicken



A

Fig. 1. IFN-γ bioassay: NO production from HD11 (A) and LSTC-IAH30 (B) cells following 48 h stimulation with supernatants from transfected COS-7 cells, or LPS (positive control — initial concentration 10 μg ml^{-1}). Supernatants tested were harvested from cells transfected with either no DNA (mock-transfected negative control), pCIneo alone (negative control), pCIneo expressing ChIFNγ or pCIneo expressing TuIFN-γ. NO production was quantified using a modification of the Griess assay, measuring absorbance at 543 nm. Error bars represent standard error of the mean.



B

Fig. 1 (*continued*).

IFN-γ neutralising Abs used were 1E-12 (mAb) and 88 (pAb). A control mAb (CC30, see Table 1), a control pAb (goat anti-mouse IgG) or no Ab at all, were also included in each assay. Test supernatants were diluted to sub-optimal levels, based on the results seen in the initial IFN-γ assays (Fig. 1).

The anti-chicken IFN-γ neutralising Abs 1E-12 and 88 both neutralised the NO-inducing activities of turkey and chicken IFN-γ on both HD11 cells and LSTC-IAH30 cells (Fig. 3). Levels of NO produced following pre-incubation with 1E-12 and 88 were significantly lower ($P < 0.001$) than those produced in all the controls, approaching the background levels seen with the pCIneo supernatants. As the rIFN-γ induced more NO production from HD11 cells than LSTC-IAH30 cells (see earlier), the neutralising effect was more obvious on HD11 cells. Neither of the control Abs had any significant effects on

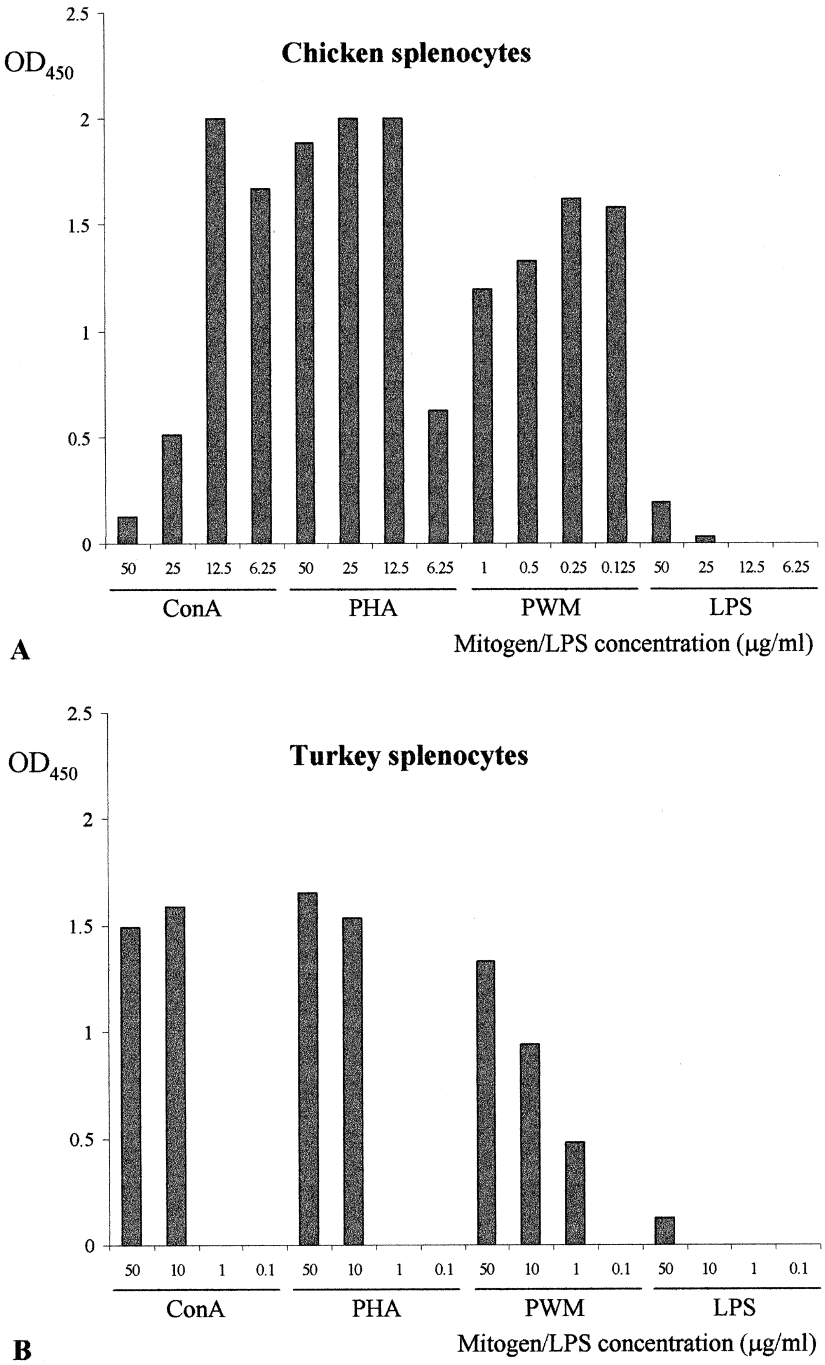


Fig. 2. IFN-γ capture ELISA: IFN-γ production from supernatants, from chicken (A) and turkey (B) splenocytes, following 48 h stimulation with various mitogens, or LPS (*E. coli* serotype 0111:B4) (positive control).

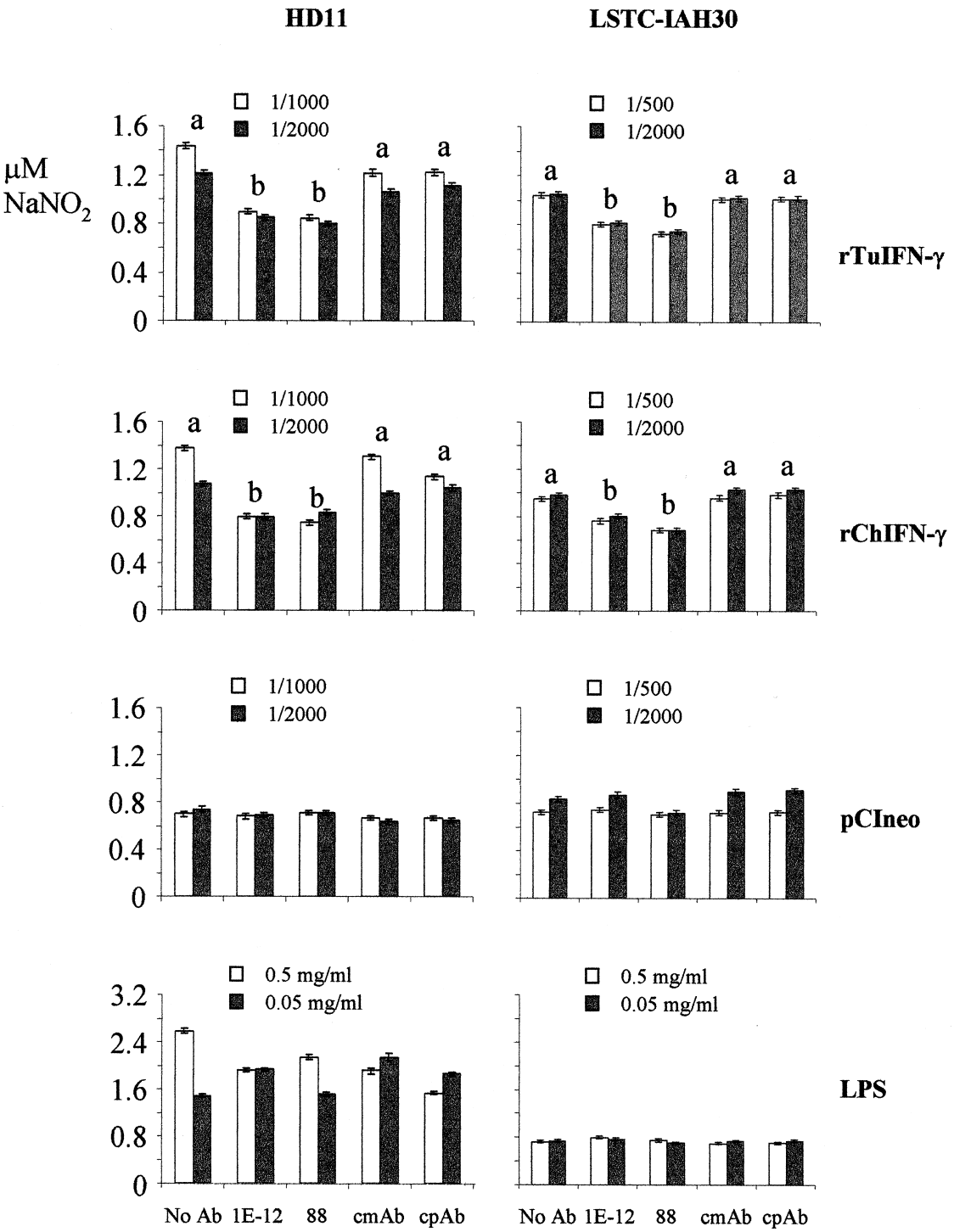
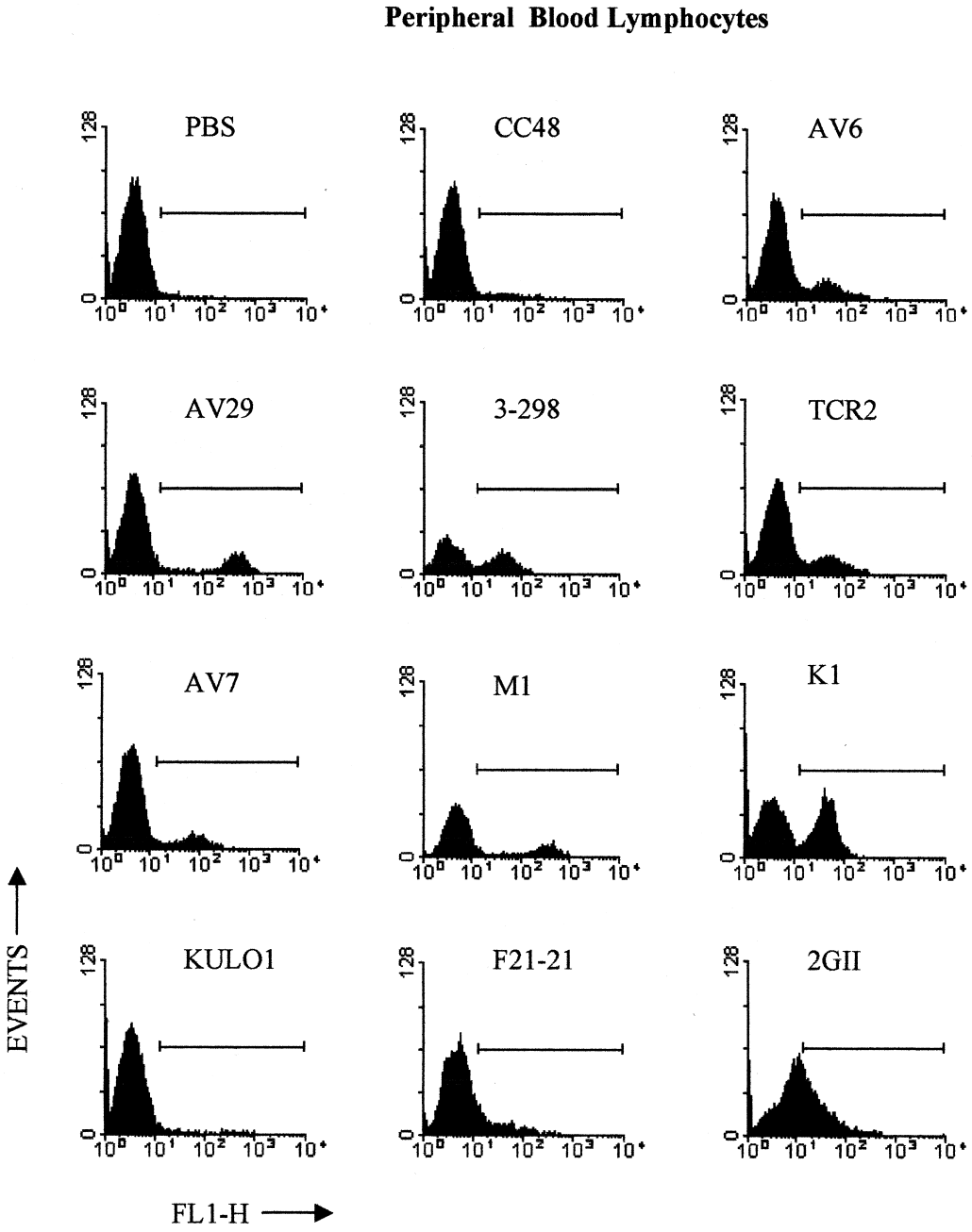


Fig. 3. IFN-γ neutralising bioassay: NO production from HD11 and LSTC-IAH30 cells following 48 h co-incubation with either IFN-γ neutralising Abs (88 and 1E12), no Ab (negative control), CC30 (control mAb) or IgG polyclonal Ab (control pAb) and either supernatants from transfected COS-7 cells (as described for Fig. 1) or LPS (*E. coli* serotype 055:B5) (positive control). Where mean values for the groups differ significantly ($P < 0.01$), the columns carry different letters. cmAb = control mAb; cpAb = control pAb.



A

Fig. 4. FACS profiles of turkey (A) PBLs and (B) splenocytes showing the cross-reactivity with a variety of fluorescently tagged anti-chicken mAb (see Table 1 for details). Negative controls were with no mAb present and an anti-bovine mAb, CC48. The *y*-axis (events) measures the number of cells present in each sample and the *x*-axis (FL1-height) measures their fluorescent intensities. The marker bar is set with reference to the control profiles and therefore indicates the cells stained positive for each test mAb.

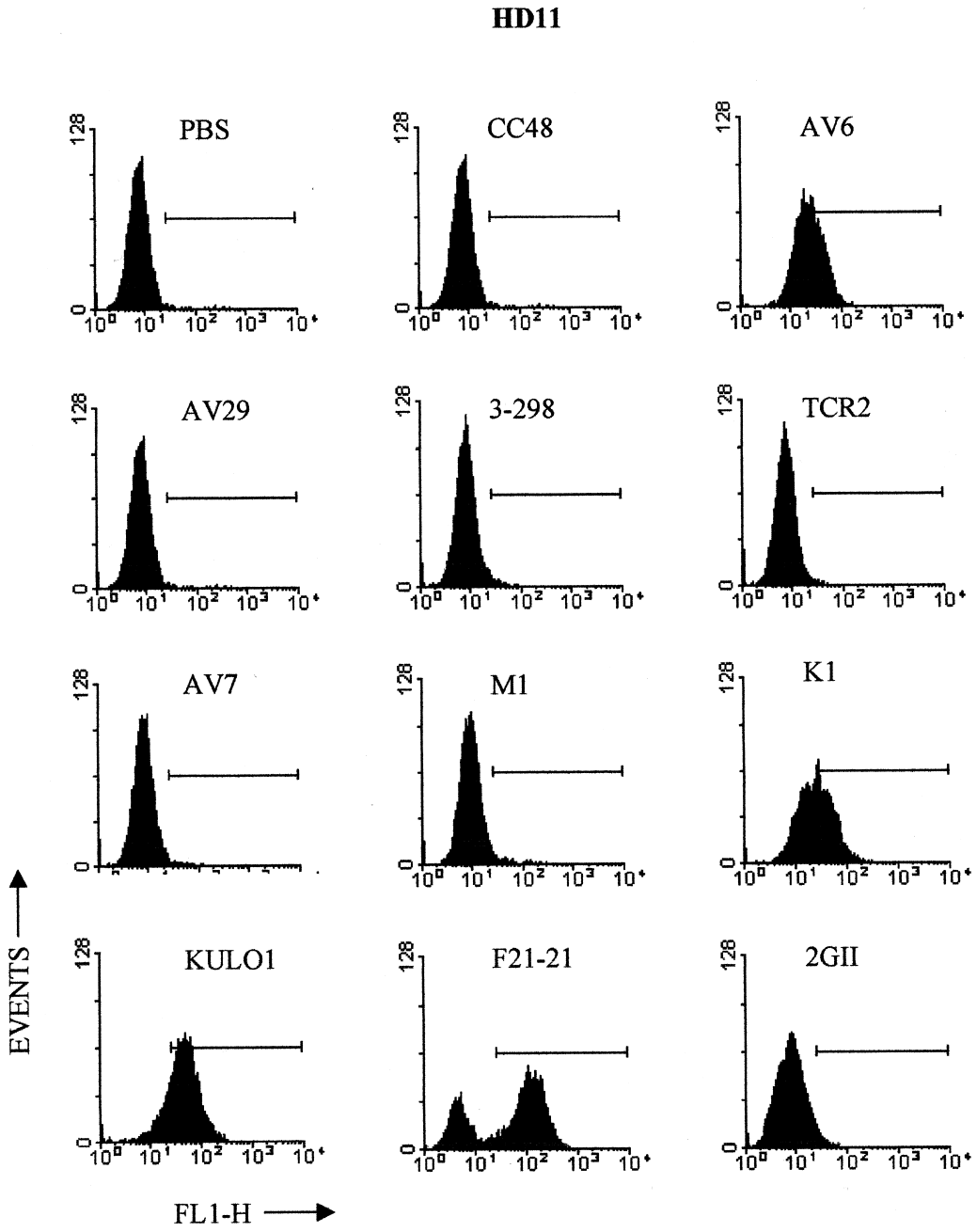


Fig. 5. FACS profiles of (A) HD11 and (B) LSTC-IAH30 cells showing the cross-reactivity with a variety of fluorescently tagged anti-chicken mAb as described in Table 1. Negative controls were with no mAb present and an anti-bovine mAb, CC30.

NO production, when compared to the no Ab controls. The presence of Ab had no effect on the levels of NO induced following incubation of either HD11 cells or LSTC-IAH30 cells with either LPS (NO assay positive control) or supernatants from COS-7 cells transfected with pCI-neo alone (NO assay negative control).

3.3. Characterisation of the expression of cell surface markers on turkey PBLs and splenocytes, and LSTC-IAH30 cells

LSTC-IAH30 cells are a previously undescribed cell line. The method used to isolate them, their morphology (data not shown) and the bioassay results suggest that they are transformed turkey macrophages. To characterise them further, a comparison of cell surface marker expression on LSTC-IAH30 and HD11 cells was carried out using flow cytometry. Initial FACS analysis showed that a number of anti-chicken mAbs (see Table 1) recognised molecules on the surface of turkey PBLs (Fig. 4(A)) and splenocytes (Fig. 4(B)). PBLs and splenocytes stained positive for all antibodies used except KULO1, which recognises a macrophage marker.

FACS analysis was carried out on HD11 and LSTC-IAH30 cells with the same panel of anti-chicken mAbs. The resulting FACS profiles are shown in Fig. 5(A) (HD11 cells) and Fig. 5(B) (LSTC-IAH30 cells). The mAbs K1 (thrombocyte/macrophage marker) and F21-21 (β_2M) stained both HD11 and LSTC-IAH30 cells, although staining was stronger with both mAbs on HD11 cells. LSTC-IAH30 cells were essentially negative for all other markers. HD11 cells were also positive for the macrophage cell surface antigen recognised by KULO1, CD44 (AV6), and MHC Class II (2G11).

4. Discussion

We had previously shown that IFN-γ shares high aa identity among the galliforms [2], which led us to predict cross-species reactivity. The data presented here conclusively show that rTuIFN-γ and rChIFN-γ are indeed cross-reactive and both can function equally well in either system. Further, mAb (1E-12) and pAb (88) raised to ChIFN-γ, which neutralise the biological activities of ChIFN-γ, also neutralise the NO-inducing activity of TuIFN-γ.

Two different cell lines were used to investigate the biological properties of rTuIFN-γ and rChIFN-γ, HD11 cells (a well-characterised chicken macrophage cell line [7,18]) and LSTC-IAH30 cells (a turkey cell line). The relative amount of NO produced by the two cell lines differed, with HD11 cells producing approximately five-fold higher maximal levels of NO than LSTC-IAH30 cells. However, the levels of NO production stimulated by either rTuIFN-γ or rChIFN-γ on either cell line were similar. Light microscopy showed that both HD11 and LSTC-IAH30 cell cultures were confluent after 48 h incubation in the presence of the rIFN-γ, arguing against LSTC-IAH30 cells having a slower growth rate than HD11 cells. The most likely explanation for the difference in induced NO levels is that the transformed turkey macrophages react less well to IFN-γ stimulation than the transformed chicken macrophages.

To further characterise the LSTC-IAH30 cell line, FACS analysis was used to compare the expression of cell surface molecules on HD11 and LSTC-IAH30 cells. Initially, turkey PBLs and splenocytes were used to determine which anti-chicken mAbs recognised epitopes on molecules on the surface of turkey cells. It should be stressed that these mAbs may not be recognising the same molecule on turkey cells as on chicken cells. There is, however, high identity between the turkey and chicken homologues of Bu-1, CD4, CD8, CD28, and CD44 (84–95% aa identity, data not shown). Further, other anti-chicken CD4 and CD8 mAbs have been shown to cross-react with turkey PBLs [19], including one of the anti-chicken CD8 mAbs used in this study, 3-298. This mAb was shown to distinguish two different populations in single-colour FACS staining, suggesting, as is the case with the chicken [20], polymorphism of the turkey molecule. Immunoprecipitation and Western blotting showed that 3-298 precipitated a 33–35 kDa polypeptide similar to the relative molecular

mass of the chicken CD8 molecule [21,22]. For the purposes of this study, we have therefore assumed that the anti-chicken mAbs recognise the same molecules on turkey cells.

HD11 cells stained positive for AV6, K1, KULO1, F21-21 and 2G11 (albeit weakly). K1 and KULO1 recognise macrophage markers, CD44 (AV6) is a widely expressed marker on both haematopoietic and non-haematopoietic cells, whilst F21-21 and 2G11 recognise class I and class II major histocompatability complex antigens respectively. LSTC-IAH30 cells were only positive for some of these markers, specifically those recognised by the mAbs K1 and F21-21, and also possibly M1 (which recognises IgM), but not KULO1. However, turkey PBLs and splenocytes were also negative for KULO1. To confirm the specificity of the mAbs used in this study, we are cloning the turkey orthologues of the respective chicken genes.

The finding that turkey and chicken IFN-γ cross-react was predicted from the high level of sequence identity. This cross-reactivity was somewhat different to the species-specificity originally observed with human IFNγ (huIFN-γ) and mouse IFN-γ [23], which share only 40% identity at the aa level [24]. More closely related mammalian species do share high identity at the predicted protein level and are cross-reactive. For example, huIFN-γ and rhesus monkey IFN-γ are 93.9% aa identical and cross-react [25]; caprine and bovine IFN-γ are 95.2% aa identical and also cross-react [26,27].

This work suggests that it may be more plausible to use rChIFN-γ, instead of TuIFN-γ, as a vaccine adjuvant against turkey diseases, because the pure recombinant chicken protein is already available and fully characterised [28–30]. Evidence for recombinant ChIFN-γ having efficacy as a vaccine adjuvant and also as an immunomodulator has recently been demonstrated [31,32]. Here we have shown cross-species reactivity between the chicken and the turkey for IFN-γ.

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